

RESEARCH

Open Access



# Phytochemical composition and anticancer effects of *Ardisia gigantifolia* butanol extract subfractions: role of cell cycle arrest and senescence in cancer cell growth inhibition

Nguyet Mai Hua<sup>1</sup>, Van Khang Pham<sup>2</sup>, Son Hiep Pham<sup>2</sup>, Thi Thanh Huong Le<sup>3</sup>, Van Hung Hoang<sup>3</sup>, Thi Kieu Oanh Nguyen<sup>4</sup>, Hong Phuong Ngo<sup>1</sup> and Phu Hung Nguyen<sup>3\*</sup>

## Abstract

**Background** The development of anticancer drugs derived from medicinal plants has recently attracted increasing attention due to advantages such as abundant natural resources and reduced side effects for patients. The present study aimed to explore the phytochemical composition and anticancer potential of butanol extract and its subfractions derived from *Ardisia gigantifolia* leaves.

**Methods and results** Using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS) analysis, 103 compounds were identified in the crude butanol extract, while 33–35 compounds were detected in each subfraction. MTT assay results showed that subfraction 5b (SubF5b) exhibited the strongest antiproliferative activity against MCF-7, MKN45, and AGS cancer cell lines, with  $IC_{50}$  values ranging from 58.2 to 73.2  $\mu\text{g/mL}$ , significantly lower than those of the crude extract and other subfractions, while showing low cytotoxicity toward normal breast epithelial MCF-10A cells. SubF5b induced G0/G1 phase arrest and pronounced cellular senescence, as evidenced by  $\beta$ -galactosidase staining. Molecular analyses showed significant downregulation of CDK4, CDK6, and CDK8, together with upregulation of the senescence markers P21 and P16 ( $p < 0.01$ ). Notably, P21 expression increased sevenfold at the mRNA level and 12-fold at the protein level. Proliferation markers Ki67 and PCNA were also significantly reduced ( $p < 0.001$ ), highlighting the potential of SubF5b as a multi-targeted anticancer agent.

**Conclusions** This study highlights the anticancer potential of *Ardisia gigantifolia*, particularly SubF5b, which exhibits strong antiproliferative and senescence-inducing effects in cancer cells. These findings support its potential as a promising candidate for the development of plant-based therapeutics targeting tumor growth and progression.

**Keywords** Breast cancer cells, Gastric cancer, *Ardisia gigantifolia*, Cell cycle, Senescence

\*Correspondence:  
Phu Hung Nguyen  
phuhungnguyen@tnu.edu.vn  
Full list of author information is available at the end of the article

## Introduction

Cancer remains one of the greatest challenges for health-care systems worldwide, with several cancer types, such as breast and gastric cancers consistently ranking among the highest in incidence and mortality rates [1]. Despite significant advancements in therapeutic approaches for both breast and gastric cancers, numerous challenges persist in prolonging patient survival, including adverse side effects, multidrug resistance, and high treatment costs [2]. Recent trends in anticancer drug development emphasize exploring the anticancer potential of plant-derived compounds, frequently in combination with synthetic chemotherapeutic agents or radiation therapy [3, 4]. This strategy aims to enhance therapeutic efficacy, reduce undesirable side effects, and alleviate economic burden on patients [5].

*Ardisia gigantifolia*, a medicinal herb indigenous to tropical and subtropical regions such as Southeast Asia and China, has long been used in traditional medicine for various health conditions. Traditional uses of *Ardisia* species include treatment for inflammatory diseases, digestive disorders, and certain stomach ailments, notably in traditional Chinese medicine [6, 7]. Prior research has revealed multiple bioactive compounds within *Ardisia gigantifolia*, including phenolics, flavonoids, alkaloids, terpenoids, and saponins [8, 9]. These phytochemicals exhibit diverse pharmacological activities, such as antimicrobial, anti-inflammatory, antioxidant, analgesic, enzyme-inhibitory, and anticancer effects [10]. Several studies have demonstrated that *Ardisia gigantifolia* possesses inhibitory effects against breast cancer, liver cancer, colon cancer and gastric cancer cells by inducing apoptosis or targeting cancer stem cells [9, 11]. For gastric cancer cells, whereas the *Ardisia gigantifolia* extract has been shown to inhibit proliferation by targeting cancer stem cells [12], silver nanoparticles synthesized from this herbal extract inhibit proliferation by inducing an overproduction of reactive oxygen species, thereby eliciting cellular stress [13]. Although specific data on the anticancer activity of the crude total ethanol extract and several isolated compounds are available, additional in-depth studies on the mechanisms and bioactivities of each distinct extract fraction are necessary. In response to these gaps, the present study evaluated the anticancer activity of the total butanol extract and its derived fractions from *Ardisia gigantifolia* against breast and gastric cancer cell lines, employing UPLC-QToF-MS-based metabolomic profiling to characterize previously unreported metabolites and systematically exploring the underlying molecular mechanisms, with particular emphasis on cell cycle regulation and activation of cellular senescence signaling pathways.

## Materials and methods

### Preparation of the butanol extract and fractions of *Ardisia gigantifolia*

*Ardisia gigantifolia* Stapf. leaves (voucher TNU2021.02) were gathered in Thai Nguyen Province, Vietnam (latitude of 21° 49' 48N, and a longitude of 105° 32' 20" E). Fresh leaves of *Ardisia gigantifolia* (3.0 kg) were thoroughly washed with tap water to remove surface impurities and subsequently air-dried under shade conditions. The dried leaf material was then subjected to extraction with 90% ethanol under reflux at 70 °C for 3 h. This process was repeated three times to maximize the extraction efficiency. The combined ethanol extracts were concentrated under reduced pressure to yield a crude ethanol extract (113.50 g).

The crude ethanol extract was subsequently suspended in 500 mL of water and partitioned successively with dichloromethane (1 L×3 times) to obtain the dichloromethane fraction and the remaining aqueous fraction [14]. The solvent from the dichloromethane fraction was removed under reduced pressure to yield the dichloromethane fraction (32.58 g). The aqueous fraction was further partitioned with *n*-butanol (1 L×3 times). The combined *n*-butanol layers were concentrated under reduced pressure to afford a *n*-butanol fraction (57.62 g). The *n*-butanol fraction was then subjected to column chromatography over silica gel, eluted with a gradient system of ethanol/dichloromethane (v/v, 1/10 to 1/1), resulting in six primary fractions (Fraction 1–6).

Fractions 2 and 5, which showed significant chemical complexity, were further purified. Fraction 2 was separated using C18 silica gel column with a mobile phase of methanol/water (v/v, 1/4–2/1), yielding five subfractions (2a–2e). Similarly, Fraction 5 was fractionated under the same conditions, yielding four subfractions (5a–5d) (Fig. 1). The *n*-butanol fractions, along with its subfractions 2b, 2d, and 5b, were selected for further purification and analysis based on preliminary MTT screening results.

### Metabolite profiling of butanol extract and subfraction of *Ardisia gigantifolia*

The metabolite profiling of the butanol extract and its subfractions of *Ardisia gigantifolia* was performed using an ACQUITY UPLC I-Class Plus system coupled with a Xevo G3 ESI/QToF high-resolution mass spectrometer (Waters Corporation, USA) [15]. Samples were prepared by dissolving 5 mg of dried extract in 0.5 mL of methanol, filtered through a 0.22 µm membrane, and 1 µL of the sample was injected for chromatographic separation using a BEH C18 column. Mass detection was conducted

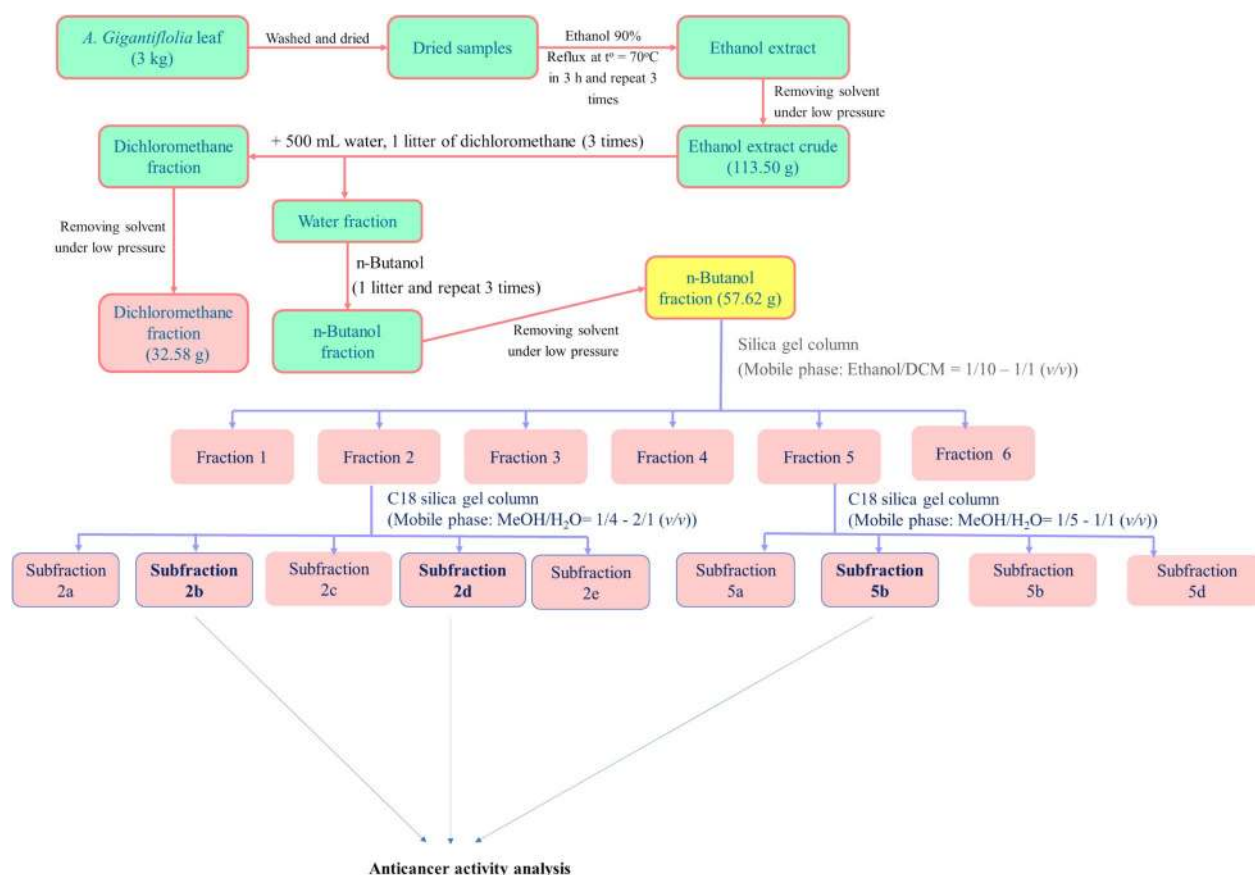


**Fig. 1** Fractionation scheme of the butanol extract from *Ardisia gigantifolia* leaves

in both positive and negative electrospray ionization modes under optimized conditions. Data acquisition was carried out in full scan mode ( $m/z$  50–1100), and fragmentation information was obtained using low- and high-energy modes. Spectral data were processed using UNIFI software, and peak annotation was performed based on fragmentation patterns compared against the Waters Traditional Medicine Library (Waters Corporation, USA).

#### Cell culture and MTT assay

MCF-7 (breast cancer cells), MKN45 and AGS (gastric cancer cells) and MCF-10A (normal breast epithelial cells) all obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), were plated in 96-well plates at  $1 \times 10^4$  cells per well in 100  $\mu$ L RPMI 1640 supplemented with 10% FBS and 1% ampicillin/streptomycin, then incubated at 37 °C in a humidified atmosphere with 5% CO<sub>2</sub>. After cell attachment (16–24 h), cultures were exposed to 0, 10, 50, 100, 200 or 500  $\mu$ g/mL of the butanol extract or its subfractions. Following 48 h of treatment, morphological changes were documented by

phase-contrast microscopy using a Nikon Ts2 inverted microscope.

The MTT assay was conducted following the previously protocol [16]. After discarding the spent medium, each well received 100  $\mu$ L of fresh medium containing MTT at 0.5 mg/mL (Thermo Fisher Scientific, USA), and was incubated at 37 °C in 5% CO<sub>2</sub> for 4 h. Thereafter, the MTT solution was removed and replaced with 100  $\mu$ L of dimethyl sulfoxide (VWR, France), followed by a 15-min incubation at 37 °C to solubilize the formazan crystals. Cell viability was assessed by measuring the absorbance at 570 nm using a Multiskan Sky spectrophotometer (Thermo Fisher Scientific, USA). Proliferation was expressed as a percentage using the formula:

$$\% \text{ Cell proliferation} = \frac{\text{OD of the treated sample}}{\text{OD of the control}} \times 100$$

All assays were conducted in triplicate across three independent experiments. IC<sub>50</sub> values for cell proliferation were determined by nonlinear regression using GraphPad Prism 10.5 in accordance with the manufacturer's guidelines.

### Cell cycle analysis

Cell cycle distribution was assessed utilizing a BD Accuri™ C6 Plus flow cytometer (BD Biosciences, USA) as previously described [17]. A total of  $2 \times 10^4$  cells were seeded in 24-well plates and incubated in RPMI 1640 medium for 24 h. Subsequently, the cells were treated with fresh medium containing a subfraction of butanol extract at varying concentrations ranging from 0 to 200  $\mu\text{g/mL}$  for a duration of 48 h. After treatment, the cells were fixed in 70% ethanol and stained with propidium iodide (PI) for 30 min. Following staining, cells were collected through trypsinization and centrifuged at 13,000 rpm for 5 min. Flow cytometric analysis was conducted to evaluate DNA content and cell cycle phases, with all experiments performed in triplicate to ensure reproducibility.

### Real-time PCR analysis for mRNA expression

MCF-7, MKN45, and AGS cancer cell lines were cultured in 6-well plates at a density of  $2 \times 10^5$  cells per well and subjected to a subfraction of the butanol extract at its  $\text{IC}_{50}$  concentration for a duration of 24 h. After treatment, total RNA was extracted utilizing TRIzol reagent (Invitrogen Thermo Fisher Scientific, US) in accordance with established protocols. Following chloroform-induced phase separation and ethanol-mediated RNA precipitation, the RNA was washed with 70% ethanol, air-dried, and reconstituted in nuclease-free water. The yield and purity of RNA were quantified using a NanoDrop spectrophotometer by evaluating the 260/280 absorbance ratio.

Quantitative real-time PCR was conducted utilizing the BIOFACT 2×One-Step SYBR Green RT-qPCR kit (BIOFACT, Korea) on a qTower<sup>3</sup> thermal cycler (Analytik Jena, Germany), with each reaction comprising 20 ng of total RNA. The thermal profile included an initial denaturation at 95 °C for 15 min, followed by 95 °C for 20 s and 60 °C for 20 s for 40 cycles. Primer sequences used are listed in Table S1. HPRT1 was used as the reference gene, and relative expression was calculated using the  $2^{-\Delta\Delta C_t}$  method [18]. All experiments were conducted in three independent replicates.

### Immunofluorescence analysis

Immunofluorescence staining was utilized to evaluate the expression of P21, PCNA, and Ki67 proteins in MKN45 gastric cancer cells as previously reported [15]. The cells were seeded in 24-well plates at a density of  $3 \times 10^4$  cells per well and allowed to adhere for 24 h. Subsequently, they were treated with 0.5 mL of fresh culture medium containing the selected subfraction at the  $\text{IC}_{50}$  concentration for 48 h under standard incubation conditions

(37 °C, 5%  $\text{CO}_2$ ). Untreated wells served as negative controls.

Following treatment, cells were fixed with 4% para-formaldehyde for 30 min and subsequently blocked in 0.3% BSA solution before incubation with primary monoclonal antibodies targeting P21 (1:500 dilution; Abcam, USA), PCNA (1:50 dilution; Santa Cruz Biotechnology, USA), and Ki67 (1:200 dilution; Thermo Fisher Scientific, USA) at room temperature for 30 min. After washing twice with PBS, the cells were incubated with Alexa Fluor 488-conjugated secondary antibodies (anti-rabbit or anti-mouse IgG, 1:200 dilution; Abcam, USA). Nuclear staining was performed using DAPI (2.5  $\mu\text{g/mL}$ , 10 min). Fluorescent signals were visualized under a Nikon Eclipse Ti2 fluorescence microscope (NIKON, Tokyo, Japan) at 20× magnification. Image acquisition and analysis were conducted using NIS-Elements software version 5.0 (NIKON, Japan).

### Data analysis

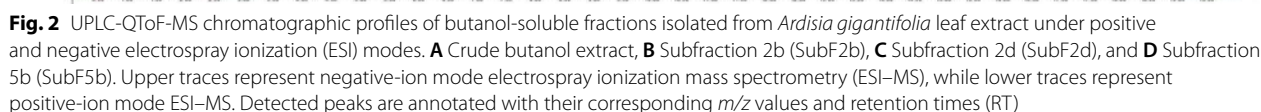
The data obtained were examined using the specialized software GraphPad Prism 10.5 (GraphPad Software, San Diego, CA, USA). The Mann–Whitney and one-way ANOVA were employed for statistical analysis, with a p-value of less than 0.05 considered as statistically significant.

## Results

### Phytochemical profiling of the butanol extract and its subfractions

The phytochemical profile of the butanol extract and its subfractions were identified utilizing UPLC-QToF-MS, with metabolite identification conducted through the Waters Traditional Medicine Library via the UNIFI software suite. Compound identification was based on precise mass determination and distinctive fragmentation patterns obtained under both low- and high-energy conditions. Chromatograms acquired in both negative and positive electrospray ionization modes for each sample of the butanol extract and its subfractions are presented in Fig. 2. Similar signal peak patterns are observed in both ionization modes. During data processing, only peaks exceeding a threshold of 15,000 counts were considered for library matching. Furthermore, the permissible mass deviation between the measured and theoretical  $m/z$  values was restricted to within  $\pm 5$  ppm.

Table 2 presents the putative annotations of the peaks satisfying the optimized criteria. The greatest number of compounds was identified in the butanol extract (102 compounds), while the subfractions SubF2b, SubF2d, and SubF5b yielded 34, 35, and 33 identified compounds,



alkaloids, diterpenoids, triterpenoids, steroids, carboxylic acids, and saponins. Eight compounds (**13**, **27**, **28**, **61**, **74**, **75**, **81**, **86** and **102**) present in SubF2b were not detected

in the other two fractions. The marker compounds distinguishing SubF2b from the other fractions are **29**, **53**, **55**, **56**, **68**, **90** and **114**. Meanwhile, SubF5b uniquely contained six compounds, including **37**, **46**, **66**, **99**, **106**, and **107**, which were not found in SubF2b or SubF2d. This heterogeneity in compound composition among the fractions may allow prediction of differences in their anticancer activity in subsequent MTT screenings.

#### **Antiproliferative activity of butanol extract and subfractions**

The results of the MTT proliferation assay (Fig. 3A–C) showed that the butanol extract and its two subfractions, SubF2b and SubF5b, all significantly reduced proliferation of all three cell lines ( $p < 0.05$ ) at concentrations starting from 50  $\mu\text{g/mL}$ . A pronounced decrease in proliferation was observed at higher concentrations (200 and 500  $\mu\text{g/mL}$ ) for both subfractions and the original butanol extract, with SubF5b exhibiting the strongest effect across all tested cell lines, reducing proliferation to 30–15% (all  $p < 0.01$ ). The  $\text{IC}_{50}$  values varied among subfractions and cell lines: SubF2b showed the highest ( $\text{IC}_{50} > 400 \mu\text{g/mL}$  for MCF-7 and MKN45, and  $> 200 \mu\text{g/mL}$  for AGS), corresponding to the lowest antiproliferative activity. In contrast, SubF5b exhibited the most potent antiproliferative effect, with  $\text{IC}_{50}$  values of 73.2, 58.2, and 62.7  $\mu\text{g/mL}$  for MCF-7, MKN45, and AGS cells, respectively. Inverted phase contrast microscopy images (Fig. 3D–F) revealed that SubF5b markedly decreased viable cell density compared to control, leaving large gaps in the culture surface. Moreover, at concentrations of 100–500  $\mu\text{g/mL}$ , pronounced morphological changes and numerous cell death phenotypes were observed, indicating a distinct cytotoxic effect of SubF5b.

To evaluate the cytotoxic effects of SubF5b on normal cells, an MTT assay was also performed on normal breast epithelial MCF-10A cells. As shown in Fig. 3G and H, a reduction in cell proliferation was observed only at concentrations of 200  $\mu\text{g/mL}$  and above, with the  $\text{IC}_{50}$  value determined to be greater than 400  $\mu\text{g/mL}$ . These MTT screening results also suggest that the SubF5b should be used for further activity analyses.

#### **Subfraction 5b induces cell cycle arrest at G0/G1 phase**

To assess the effect of SubF5b on cell cycle progression, flow cytometry analysis was performed on all three

cancer cell lines. Figure 4 demonstrates a significant alteration in the distribution of cells across the cell cycle phases following treatment with SubF5b. Specifically, an accumulation of cells in the G0/G1 phase was observed across all three cell lines: MCF-7 (Fig. 4A, D), MKN45 (Fig. 4B, E), and AGS (Fig. 4C, F). The percentage of cells in this phase increased to 60–65%, compared to 55–58% in the control groups ( $p < 0.05$ ), with variations depending on the specific cell line. Correspondingly, the proportion of cells in the G2/M phase decreased. Thus, SubF5b induced G0/G1 arrest, thereby disrupting progression through the cell cycle.

#### **Subfraction 5b induces cell senescence**

Cell cycle arrest at G0/G1 suggested an effect of SubF5b on cellular senescence.  $\beta$ -Galactosidase staining, a canonical marker of senescence, was evaluated (Fig. 5A–C), revealing induction of senescence by SubF5b in all three cell lines, with magnitude varying by line. Senescent cells, which were stained blue due to the turnover of X-gal by  $\beta$ -galactosidase, were observed at all tested concentrations (50, 100, and 200  $\mu\text{g/mL}$ ). The highest density of senescent cells was observed at 50  $\mu\text{g/mL}$  SubF5b, decreasing at 100 and 200  $\mu\text{g/mL}$ . ImageJ quantification (Fig. 5D–F) showed the proportion of senescent cells rising from 1% in controls to approximately 5–70%, depending on concentration and cell line ( $p < 0.01$ ). At high concentrations of 100 and 200  $\mu\text{g/mL}$  of SubF5b, the majority of cells observed had a dead cell appearance.

#### **Subfraction 5b affects on gene expression of cell cycle and senescence signaling**

To further elucidate the impact of SubF5b on cell cycle arrest, transcriptional-level analyses by real-time PCR and protein expression profiling by immunofluorescence were performed (Fig. 6). The gene expression analysis at the transcriptional (mRNA) level in Fig. 6A demonstrated that SubF5b significantly downregulated cell cycle-related genes, including CDK4, CDK6, and CDK8, while no notable changes were observed in CDK2 and CDK3 expression. Genes closely associated with cellular senescence, including P21, P16, Ki67, and PCNA, also exhibited significant transcriptional alterations. Specifically, P21 expression increased

(See figure on next page.)

**Fig. 3** Effects of butanol extract and its subfractions on the proliferation of cancer cells. **A** MCF-7 breast cancer, **B** MKN45, and **C** AGS gastric cancer cells. Data are presented as the mean  $\pm$  standard deviation ( $n=4$ ), Mann–Whitney test, \* $p < 0.05$ , \*\* $p < 0.01$  compared to the control; effects of SubF5b on cancer cell morphology (**D–E**): **D** MCF-7, **E** MKN45, and **F** AGS cells; and **G, H** effects of SubF5b on the proliferation and cell morphology of normal breast epithelial cells MCF-10A. Data are presented as the mean  $\pm$  standard deviation ( $n=4$ ), Mann–Whitney test, \* $p < 0.05$ , \*\* $p < 0.01$  compared to the control. Scale bar = 90  $\mu\text{m}$

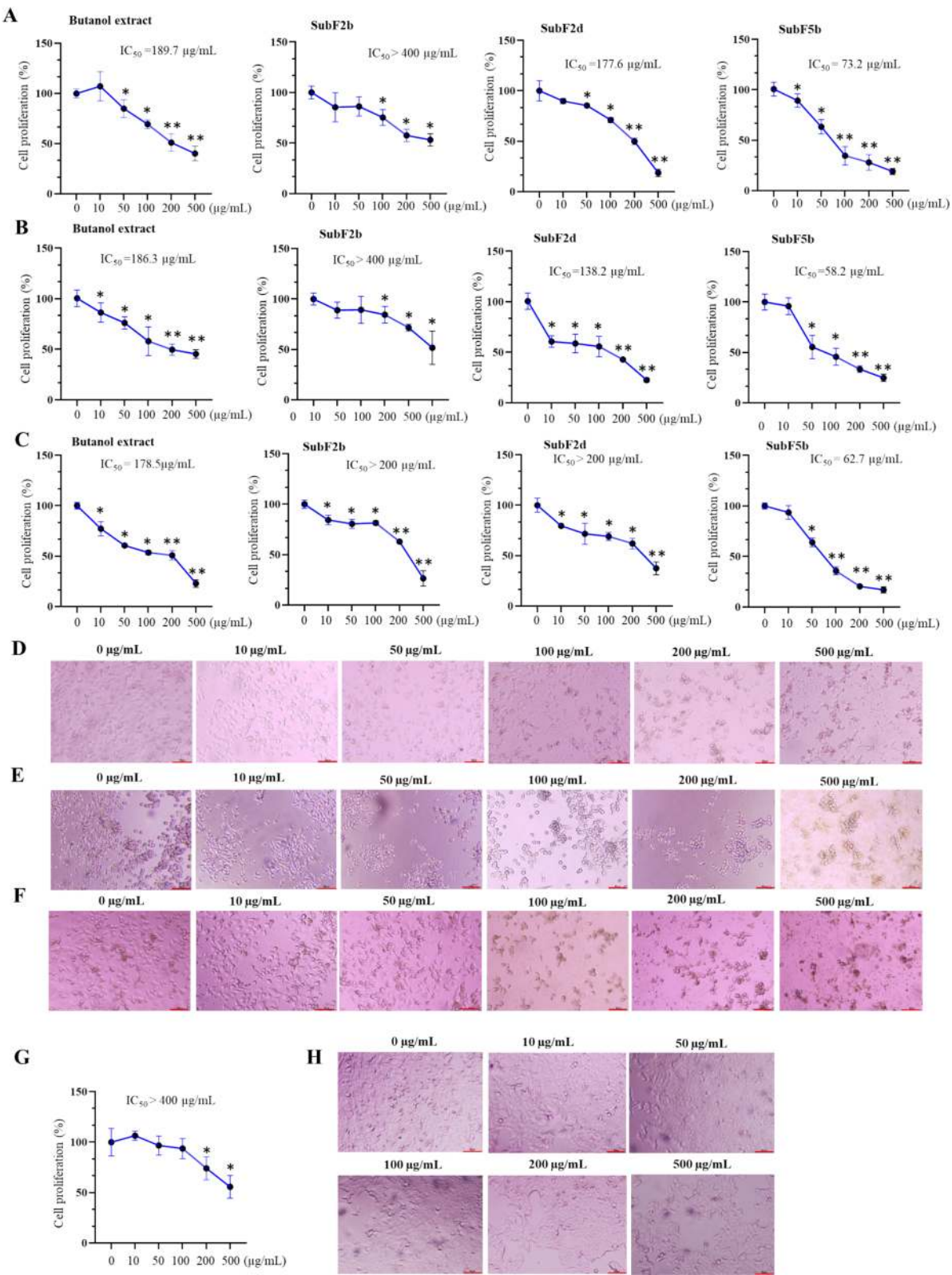


**Fig. 3** (See legend on previous page.)

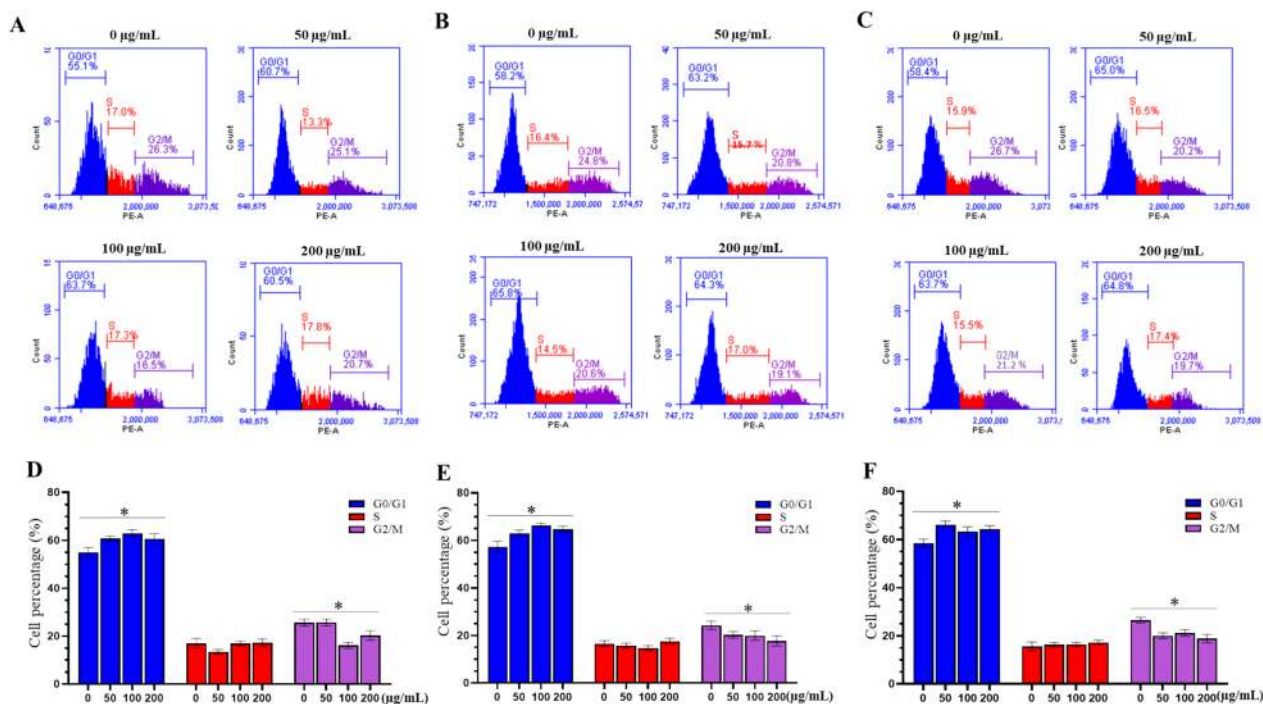


**Fig. 4** Effects of SubF5b on the cell cycle. Cells were treated with SubF5b at concentrations of 0, 50, 100 and 200 µg/mL, stained with PI, and analyzed using flow cytometry: **A, D** MCF-7 breast cancer cells, **B, E** MKN45 and (**C, F**) AGS gastric cancer cells. Data are presented as the mean  $\pm$  standard deviation ( $n = 3$ ), ANOVA test, \*  $p < 0.05$

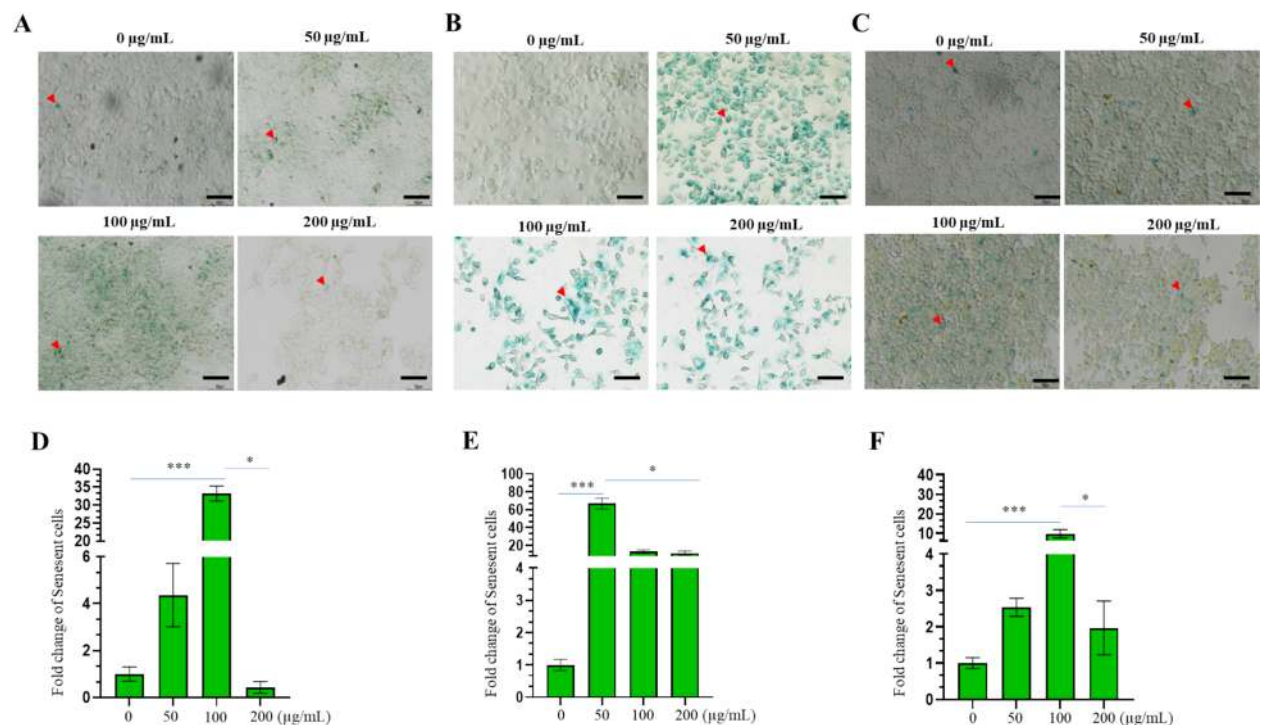


**Fig. 5** Effects of subfractions 5b (SubF5b) on cell senescence. Cancer cells were treated different concentration of SubF5b (0, 50, 100 or 200 µg/mL). Images and percentage of senescent cells were captured under an inverted microscope at 100 $\times$  magnification: **A, D** MCF-7 breast cancer, **B, E** MKN45 and **C, F** AGS gastric cancer cells. Senescent phenotype cells. Scale bar = 90 µm. Data are presented as the mean  $\pm$  standard deviation ( $n = 3$ ). ANOVA test, \*\*\*  $p < 0.001$  versus control

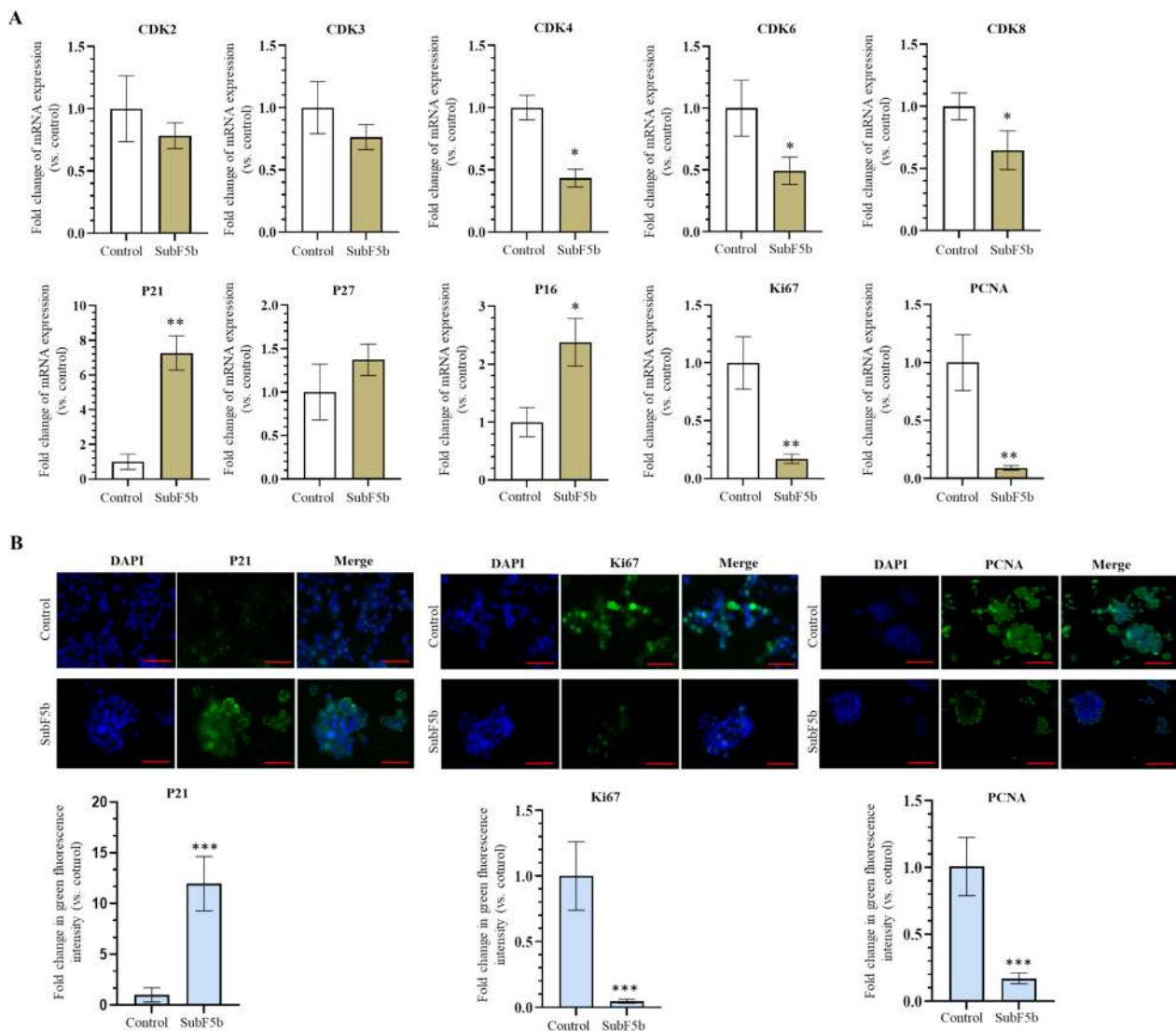


**Fig. 6** Effects of SubF5b on the expression of cell cycle- and senescence-related genes of MKN45 cells. Cancer cells were treated with extracts at  $IC_{50}$  concentrations (58.2  $\mu$ g/mL) for 24 h, and were then analyzed by real-time PCR (**A**). Changes in mRNA levels of genes were analysis by  $2^{-\Delta\Delta Ct}$  method. Expression of protein P21, Ki67 and PCNA were analyzed by immunofluorescence (**B**). Cell nuclei were stained with DAPI (blue), and P21-, Ki67-, or PCNA-positive cells appeared green. All results are presented as mean  $\pm$  SD ( $n=3$ ). Mann–Whitney test, \* $p<0.05$ , \*\* $p<0.01$

approximately sevenfold compared to the control ( $p<0.01$ ), whereas P16 showed a 2.5-fold upregulation ( $p<0.05$ ). In contrast, Ki67 and PCNA expression decreased by approximately tenfold and fivefold, respectively ( $p<0.01$ ). No other significant changes in mRNA levels were detected.

Protein expression was analyzed using immunofluorescence (Fig. 6B) revealed a robust increase in P21 levels (12-fold,  $p<0.001$ ), while Ki67 and PCNA exhibited marked reductions (20-fold and eightfold, respectively, all  $p<0.001$ ). These findings indicate that SubF5b modulates

the expression of key genes involved in cell cycle regulation and cellular senescence pathways.

## Discussion

The increasing global incidence of cancer, particularly breast and gastric malignancies, presents significant challenges in developing novel therapeutic agents that can effectively eliminate tumor cells while circumventing drug resistance, minimizing adverse effects, and ensuring cost-effectiveness. This necessity has redirected research efforts towards the investigation of traditional and herbal medicines, which have historically demonstrated efficacy

against various pathological conditions [19]. In this study, we present the first comprehensive characterization of the principal constituents of butanol extract and its fractions using ultra-performance liquid chromatography coupled with high-resolution quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS).

Comprehensive metabolite profiling not only reveals the chemical composition of the butanol extract and its subfractions, which is useful for chemotaxonomy but could also be useful for determining the bioactive compounds. In this research, we identified the presence of 103 different compounds in the butanol extract, and 34–35 compounds in each of its subfractions. Notably, 17 different compounds were found in all the subfractions and the extract, which may give rise to similar biological properties among the subfractions. In which, Nonadecanoic acid (compound **79**), isolated from *Ganoderma lucidum*, exhibited inhibitory activity and induced apoptosis against the proliferation of HL-60 leukemia cells, with an  $IC_{50}$  value of  $68 \pm 7 \mu M$  [20]. Magnoflorine (compound **80**), an alkaloid isolated from *Berberis cretica* roots, exhibits antiproliferative and pro-apoptotic effects, and caused cell cycle arrest at S/G2 phases in lung cancer (NCI-H1299), MDA-breast cancer (MB-468) in a dose-dependent manner, indicating its potential as a promising anticancer agent [21]. Other compound, such as phytolaccagenic acid (compound **92**), have also been reported to exhibit anticancer activity against gastric cancer cell line SGC-7901 and liver cancer cell line HepG2 [22].

However, the presence of 6–8 unique compounds in each subfraction may lead to differences in biological properties, as exemplified by the anticancer activity on different cell lines. A previous report showed that fractions from *Phoenix dactylifera* contain various volatile compounds identified by GC-MS. These fractions also exhibited different inhibitory effects on pancreatic cancer cell lines PT45P1, PANC-1, and SUII-2 [23]. In another study, **81** and **100** compounds were identified in the extract fractions of *Ophiocordyceps xuefengensis* and *Ophiocordyceps sinensis*, respectively, of which **68** were common to both species. Subsequently, the fractions of each species displayed different levels of cancer cell inhibition in esophageal and breast cancer cell lines, monocytic leukemia, and Burkitt's lymphoma [24]. Previously, molecular docking analysis showed that podophyllotoxin (compound **4**) has a strong binding affinity to the human estrogen receptor  $\alpha$  (binding energy =  $-8.38$  kcal/mol), indicating its potential as an anti-breast cancer agent [25]. Meanwhile, evodiamine (compound **5**) demonstrated inhibitory activity against the PI3Kp110 protein, a component of the PI3K/AKT signaling pathway, leading to the induction of apoptosis in small-cell lung cancer cell lines NCI-H446 and

NCI-H1688 [26]. Similarly, based on molecular docking analysis, limonin was suggested to have inhibitory potential toward the VEGFR2 receptor, resulting in suppressed proliferation and migration of breast cancer cells (MDA-MB-231, MCF-7) [27]. The chemical composition of the *n*-butanol extract subfractions of *Kigelia africana* was also analyzed using UPLC-QToF-MS, revealing the presence of over 200 identified compounds, which exhibited cytotoxic activity against HepG2 and HeLa cancer cell lines [28]. The results of the antiproliferative activity assay (Fig. 3) on breast and gastric cancer cell lines revealed distinct differences among the butanol extract subfractions tested. Notably, SubF5b exhibited the lowest  $IC_{50}$  values ( $73.2$ – $58.2 \mu g/mL$ ), indicating that this fraction possessed the most potent anticancer activity in all three cancer cell lines examined, while exerting only low cytotoxic effects on normal breast epithelial MCF-10A cells ( $IC_{50} > 400 \mu g/mL$ ). These variations may be attributed to the composition of each subfraction.

A previous study investigating the anticancer effects of *Ardisia crispa*—a species from the same genus as *Ardisia gigantifolia*—reported that both the hydromethanolic and ethyl acetate extracts exhibited significant antiproliferative activity against MCF-7 breast cancer cells, with  $IC_{50}$  values of  $57.35 \pm 19.33 \mu g/mL$  and  $54.98 \pm 14.10 \mu g/mL$ , respectively. In contrast, the aqueous extract of the same plant showed much weaker activity, with an  $IC_{50}$  value exceeding  $1000 \mu g/mL$  [29]. Notably, a study on *Ardisia gigantifolia* revealed that extracts prepared using different solvents exhibited distinct cytotoxic effects against HCT116 colorectal cancer cells. Specifically, the *n*-butanol extract showed an  $IC_{50}$  value of  $197.24 \mu g/mL$ , while the ethyl acetate and petroleum ether fractions had  $IC_{50}$  values of  $264.85 \mu g/mL$  and  $15.45 \mu g/mL$ , respectively. The underlying inhibitory mechanism is indicated to be the targeting of MAPK1, ESRI, HSP90AA1, and MAPK8 proteins by compounds present in the extracts of *Ardisia gigantifolia*. These results indicate that different solvents, by extracting different sets of compounds, can lead to variable anticancer activities against cancer cell lines [30].

The progression of cells through the various phases of the cell cycle is essential for maintaining stable and continuous cellular proliferation over time. Disruptions in this tightly regulated process, particularly interventions that hinder cell cycle progression, can impair cell viability and reduce proliferative capacity. This effect is currently being exploited as a therapeutic strategy for cancer treatment. In this study, the impact of SubF5b on the cell cycle was assessed using flow cytometry (Fig. 4), which revealed a marked arrest in the G0/G1 phase. This accumulation of cells in G0/G1 was accompanied by a

concomitant reduction in the proportion of cells in the G2/M phase, suggesting a mechanism by which SubF5b suppresses cellular proliferation in MCF-7, MKN45, and AGS cancer cell lines compared to untreated controls.

Previous studies have similarly demonstrated that subfractions derived from the butanol extract of *Brasica rapa* inhibited the proliferation of A549 lung cancer cells by inducing G0/G1 cell cycle arrest through a ROS-dependent mechanism [31]. In contrast, n-butanol fractions from *Avena sativa* were shown to inhibit the proliferation of HepG2 liver cancer cells by inducing arrest at the S and G2/M phases [32]. The phase-specific effects of these extracts may depend on the phytochemical composition of the fractions and the botanical species from which they are derived.

In the present study, the G0/G1 arrest induced by SubF5b provides mechanistic insight that may relate to its ability to promote cellular senescence. Indeed, previous reports have linked cancer cell senescence with G0/G1 arrest, particularly through the upregulation of the cyclin-dependent kinase inhibitors P21 and P16 [33], both of which were significantly increased following SubF5b treatment in our model.

The findings of this study demonstrate that SubF5b extracted from *Ardisia gigantifolia* exerts a significant anti-proliferative effect by inducing cellular senescence in breast (MCF-7) and gastric cancer cell lines (MKN45 and AGS). Senescence was confirmed through enhanced  $\beta$ -galactosidase-positive staining, a well-established marker of cellular senescence. These observations align with previous studies reporting that plant-derived extracts, such as *Elsholtzia winitiana*, can induce senescence via cell cycle arrest-related mechanisms in AGS gastric cancer [34]. In other studies, ginger extract and *Olea europaea* L. leaf extract induced senescence through telomere shortening at chromosomal termini in A549 lung cancer [35] and glioblastoma [36].

A significant finding of this study is that the senescence-inducing effect was most pronounced at the lower concentration of 50  $\mu\text{g/mL}$  and progressively diminished at higher concentrations (100 and 200  $\mu\text{g/mL}$ ). This outcome may indicate a concentration-dependent shift in the cellular response, wherein higher doses of the extract potentially activate alternative cell death pathways, such as apoptosis or necrosis. Similar phenomenon has been previously documented in the context of the chemotherapeutic agent doxorubicin in MCF-7 breast cancer cells [37], as well as with other anticancer agents across diverse cancer models [38]. Understanding such dose-dependent cellular dynamics is crucial for the rational development and therapeutic optimization of herbal extracts and isolated phytochemicals for cancer treatment.

Among the compounds identified in the SubF5b fraction (Table 1 and Table S2), several were present in high abundance, including Brucine (compound 63), 16-Methyl-heptadecanoic acid (compound 84), Physanol B (compound 105), Gossypitrin (compound 99), and Dibutyl sebacate (compound 66) (Table 2). Previous studies have demonstrated that Brucine inhibits the growth of cervical cancer cells by inducing apoptosis through the PI3K/AKT/mTOR signaling pathway [39]. The marine fungal metabolite 16-methyl-heptadecanoic acid methyl ester was shown to inhibit the proliferation of oral cancer KB cells and skin carcinoma A431 cells, and it also induced ROS-dependent apoptosis, downregulated heat shock protein 90, and exhibited potent in vivo anti-skin cancer activity [40]. Meanwhile, Physanol B has been reported to suppress HGC-27 cell proliferation in a dose- and time-dependent manner, trigger G0/G1 arrest and caspase-dependent apoptosis, upregulate cleaved caspases and p-Chk2, while downregulating cyclins, CDKs, and phosphorylated Rb protein [41]. In addition, Gossypetin isolated from *Hibiscus sabdariffa* was shown to induce cell cycle arrest and promote apoptosis by activating NF-kappa B in oral cancer [42].

In addition, several compounds present at lower abundance, such as compounds 42, 43, 44, 102, 83, and 86, have also been reported to exhibit potent anticancer activities. Sanggenon C (compound 101) has been reported to exhibit anti-proliferative activity in K562 leukemic cells by inducing G0/G1 cell cycle arrest [43], as well as in PC3 and DU145 prostate cancer cells [44]. Meanwhile, Xanthones (compound 85) have been documented to trigger G1-phase cell cycle arrest in colon cancer cell lines [45]. Additionally, Coixenolide (compound 83), has demonstrated inhibitory effects on cervical cancer through various mechanisms, such as apoptosis induction or targeting the AKT/mTOR signaling pathway [42]. Although these metabolites (compounds 102, 86, 63, and 84) have been reported to exhibit anticancer activity against various cancer cell lines, their presence has not been previously documented in studies on *Ardisia gigantifolia*. Other compounds present in SubF5b may not yet have reported anti-cancer activity or mechanisms related to senescence. However, the present study does not establish a direct causal relationship between any single compound and the observed bioactivity. In future studies, the isolation and direct evaluation of these compounds, particularly Brucine and Gossypitrin on cancer cells will be essential to further elucidate their mechanisms of action.

The expression levels of CDKs play a crucial role in cell cycle regulation, with CDK4/CDK6 being essential for cell cycle progression from G0/G1 to S phase [46].

**Table 1** Phytochemical compounds of Butanol extract and fractions from *Ardisia gigantifolia* leaves

| No | Compounds                            | Formular                                          | RT(min)  | Butanol extract | SubF2b | SubF2d | SubF5b | Classification  |
|----|--------------------------------------|---------------------------------------------------|----------|-----------------|--------|--------|--------|-----------------|
| 1  | Mahuannin G                          | C <sub>30</sub> H <sub>22</sub> O <sub>10</sub>   | 0.62536  |                 |        | ✓      |        | Flavonoid       |
| 2  | Decursitin F                         | C <sub>16</sub> H <sub>16</sub> O <sub>6</sub>    | 0.66422  | ✓               |        |        |        | Coumarin        |
| 3  | Bruceine G                           | C <sub>20</sub> H <sub>26</sub> O <sub>8</sub>    | 0.66778  | ✓               |        |        |        | Triterpenoid    |
| 4  | Podophyllotoxin                      | C <sub>22</sub> H <sub>22</sub> O <sub>8</sub>    | 0.67046  | ✓               |        |        |        | Lignan          |
| 5  | Evodiamine                           | C <sub>19</sub> H <sub>17</sub> N <sub>3</sub> O  | 0.67416  |                 |        | ✓      |        | Alkaloid        |
| 6  | Kushenol G                           | C <sub>25</sub> H <sub>28</sub> O <sub>8</sub>    | 0.7376   | ✓               |        |        |        | Flavonoid       |
| 7  | Didemthoxylcurcumin                  | C <sub>19</sub> H <sub>16</sub> O <sub>4</sub>    | 0.97649  | ✓               |        |        |        | Phenolic        |
| 8  | Epigallocatechin(4β,8)-gallocatechin | C <sub>30</sub> H <sub>26</sub> O <sub>14</sub>   | 1.31246  |                 |        | ✓      | ✓      | Flavonoid       |
| 9  | Linderazulene                        | C <sub>15</sub> H <sub>14</sub> O                 | 1.52084  | ✓               | ✓      |        | ✓      | Terpenoid       |
| 10 | 4',7-Dimethylctori-genin             | C <sub>18</sub> H <sub>16</sub> O <sub>6</sub>    | 1.74999  | ✓               |        | ✓      | ✓      | Flavonoid       |
| 11 | Buddlenoid A                         | C <sub>30</sub> H <sub>26</sub> O <sub>13</sub>   | 1.76553  | ✓               |        |        |        | Flavonoid       |
| 12 | Auraptenol                           | C <sub>19</sub> H <sub>22</sub> O <sub>4</sub>    | 4.18247  | ✓               | ✓      | ✓      | ✓      | Coumarin        |
| 13 | 8-Hydroxy-dihydrochelyerythrine      | C <sub>21</sub> H <sub>19</sub> NO <sub>5</sub>   | 5.60539  | ✓               | ✓      |        |        | Alkaloid        |
| 14 | Kansuinin B                          | C <sub>38</sub> H <sub>42</sub> O <sub>14</sub>   | 7.12650  | ✓               |        |        |        | Terpenoid       |
| 15 | Corylin                              | C <sub>20</sub> H <sub>16</sub> O <sub>4</sub>    | 7.55533  | ✓               |        |        |        | Flavonoid       |
| 16 | Saucernetin-7                        | C <sub>41</sub> H <sub>48</sub> O <sub>11</sub>   | 7.64040  | ✓               |        |        |        | Lignan          |
| 17 | N,N'-Dicarbazyl                      | C <sub>24</sub> H <sub>16</sub> N <sub>2</sub>    | 7.67739  | ✓               |        |        |        | Alkaloid        |
| 18 | γ-Linoleic acid                      | C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>    | 7.91843  | ✓               | ✓      |        | ✓      | Carboxylic acid |
| 19 | Daturametelin I                      | C <sub>34</sub> H <sub>48</sub> O <sub>10</sub>   | 7.91903  | ✓               |        |        |        | Steroid         |
| 20 | Limocitrin-3,7-O-β-D-glucopyranoside | C <sub>29</sub> H <sub>34</sub> O <sub>18</sub>   | 7.9224   | ✓               |        |        |        | Glycoside       |
| 21 | Lappaol D                            | C <sub>31</sub> H <sub>36</sub> O <sub>10</sub>   | 7.92273  | ✓               |        |        |        | Flavonoid       |
| 22 | Taurocholic acid                     | C <sub>26</sub> H <sub>45</sub> NO <sub>7</sub> S | 7.92553  | ✓               |        |        |        | Steroid         |
| 23 | Marstenacigenin A                    | C <sub>30</sub> H <sub>42</sub> O <sub>6</sub>    | 7.92570  | ✓               |        |        |        | Steroid         |
| 24 | 6α-Acetoxy-5-epilimonin              | C <sub>30</sub> H <sub>38</sub> O <sub>9</sub>    | 7.92577  | ✓               |        |        |        | Limonoid        |
| 25 | Lactucin                             | C <sub>15</sub> H <sub>16</sub> O <sub>5</sub>    | 7.92672  | ✓               |        |        |        | Terpenoid       |
| 26 | Evoprenine                           | C <sub>20</sub> H <sub>21</sub> NO <sub>4</sub>   | 8.14039  | ✓               |        |        |        | Alkaloid        |
| 27 | Kansuiphorin C                       | C <sub>29</sub> H <sub>34</sub> O <sub>6</sub>    | 8.29132  | ✓               | ✓      |        |        | Terpenoid       |
| 28 | Magnaldehyde B                       | C <sub>18</sub> H <sub>16</sub> O <sub>3</sub>    | 8.54916  | ✓               | ✓      |        |        | Lignan          |
| 29 | Methyl ganoderate E                  | C <sub>33</sub> H <sub>46</sub> O <sub>9</sub>    | 8.69972  | ✓               |        | ✓      |        | Triterpenoid    |
| 30 | 19β-Glucosylandrographoside          | C <sub>26</sub> H <sub>40</sub> O <sub>10</sub>   | 8.72238  | ✓               | ✓      |        | ✓      | Glycoside       |
| 31 | Coronaric acid                       | C <sub>18</sub> H <sub>32</sub> O <sub>3</sub>    | 9.24366  | ✓               | ✓      | ✓      | ✓      | Carboxylic acid |
| 32 | Platycodigenin                       | C <sub>30</sub> H <sub>48</sub> O <sub>7</sub>    | 9.42111  | ✓               |        |        |        | Triterpenoid    |
| 33 | Meranzin hydrate                     | C <sub>15</sub> H <sub>18</sub> O <sub>5</sub>    | 9.48552  | ✓               |        |        |        | Coumarin        |
| 34 | Bis-andrographolide C                | C <sub>40</sub> H <sub>56</sub> O <sub>8</sub>    | 9.49864  | ✓               | ✓      | ✓      | ✓      | Terpenoid       |
| 35 | Methyl lucidenate P                  | C <sub>30</sub> H <sub>44</sub> O <sub>8</sub>    | 9.65365  | ✓               |        |        |        | Triterpenoid    |
| 36 | Pterodontoside H                     | C <sub>21</sub> H <sub>36</sub> O <sub>7</sub>    | 9.65680  | ✓               |        |        |        | Terpenoid       |
| 37 | Sophoranodichromane A                | C <sub>25</sub> H <sub>28</sub> O <sub>6</sub>    | 9.68383  |                 |        |        | ✓      | flavanonol      |
| 38 | Ambrettolid                          | C <sub>16</sub> H <sub>28</sub> O <sub>2</sub>    | 9.88559  | ✓               | ✓      | ✓      | ✓      | Ester           |
| 39 | Scutellone I                         | C <sub>20</sub> H <sub>26</sub> O <sub>5</sub>    | 9.90679  | ✓               | ✓      | ✓      | ✓      | Diterpenoid     |
| 40 | Picrasinoside G                      | C <sub>28</sub> H <sub>44</sub> O <sub>12</sub>   | 9.93452  | ✓               |        |        |        | Triterpenoid    |
| 41 | Picrasinoside G                      | C <sub>28</sub> H <sub>44</sub> O <sub>12</sub>   | 9.93452  | ✓               |        |        |        | Triterpenoid    |
| 42 | Ginsenoside F3                       | C <sub>41</sub> H <sub>70</sub> O <sub>13</sub>   | 10.02715 | ✓               |        |        |        | Triterpenoid    |
| 43 | Nomilin                              | C <sub>28</sub> H <sub>34</sub> O <sub>9</sub>    | 10.13676 | ✓               |        |        |        | Triterpenoid    |
| 44 | Epiberberine                         | C <sub>20</sub> H <sub>18</sub> NO <sub>4</sub>   | 10.14685 | ✓               |        |        |        | Alkaloid        |
| 45 | Sinkianone                           | C <sub>24</sub> H <sub>30</sub> O <sub>4</sub>    | 10.20392 | ✓               |        |        |        | Coumarin        |
| 46 | Decursidin                           | C <sub>24</sub> H <sub>26</sub> O <sub>7</sub>    | 10.21548 | ✓               |        |        | ✓      | Coumarin        |
| 47 | Ricinoleic acid                      | C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>    | 10.63613 | ✓               |        | ✓      | ✓      | Carboxylic acid |
| 48 | Feterin                              | C <sub>26</sub> H <sub>32</sub> O <sub>6</sub>    | 10.68646 | ✓               |        |        |        | Coumarin        |
| 49 | Chikusetsusaponin Ia                 | C <sub>41</sub> H <sub>70</sub> O <sub>2</sub>    | 10.71223 | ✓               |        |        |        | Steroid         |

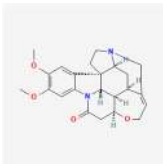
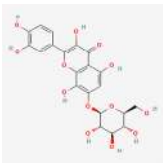
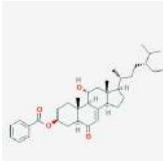
**Table 1** (continued)

| No | Compounds                                | Formular                                                      | RT(min)  | Butanol extract | SubF2b | SubF2d | SubF5b | Classification  |
|----|------------------------------------------|---------------------------------------------------------------|----------|-----------------|--------|--------|--------|-----------------|
| 50 | Lignoceryl ferulate                      | C <sub>34</sub> H <sub>58</sub> O <sub>4</sub>                | 10.71280 | ✓               | ✓      | ✓      | ✓      | Ester           |
| 51 | Palmitelaidic acid                       | C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>                | 10.73322 | ✓               | ✓      | ✓      |        | Carboxylic acid |
| 52 | Daturametelin D                          | C <sub>29</sub> H <sub>40</sub> O                             | 10.83451 | ✓               |        |        |        | Steroid         |
| 53 | Panaxynol                                | C <sub>17</sub> H <sub>24</sub> O                             | 10.87685 |                 |        | ✓      |        | Alcohol         |
| 54 | Marsdenoside B                           | C <sub>45</sub> H <sub>68</sub> O <sub>14</sub>               | 10.89421 | ✓               |        |        |        | Glycoside       |
| 55 | Kansuiphorin A                           | C <sub>54</sub> H <sub>90</sub> O <sub>9</sub>                | 11.04580 | ✓               |        | ✓      |        | Terpenoid       |
| 56 | Pseudobrucine                            | C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>5</sub> | 11.06526 |                 |        | ✓      |        | Alkaloid        |
| 57 | ent-Kauran-16β,17-diol                   | C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>                | 11.08018 | ✓               | ✓      | ✓      |        | Alcohol         |
| 58 | Galloyloxypaeoniflorin                   | C <sub>30</sub> H <sub>32</sub> O <sub>16</sub>               | 11.09690 | ✓               |        |        |        | Polyphenol      |
| 59 | Lauryl acetate                           | C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>                | 11.16046 | ✓               | ✓      | ✓      | ✓      | Ester           |
| 60 | Yesanchinoside E                         | C <sub>54</sub> H <sub>92</sub> O <sub>23</sub>               | 11.22322 | ✓               |        |        |        | Glycoside       |
| 61 | Astragaloside IV                         | C <sub>41</sub> H <sub>68</sub> O <sub>14</sub>               | 11.32166 | ✓               | ✓      |        |        | Glycoside       |
| 62 | Kuwanon E                                | C <sub>25</sub> H <sub>28</sub> O <sub>6</sub>                | 11.52191 | ✓               |        |        |        | Flavonoid       |
| 63 | Brucine                                  | C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> | 11.53445 | ✓               | ✓      | ✓      | ✓      | Alkaloid        |
| 64 | Dihydropalmitine                         | C <sub>21</sub> H <sub>23</sub> NO <sub>4</sub>               | 11.54077 | ✓               |        |        |        | Alkaloid        |
| 65 | Licochalcone A                           | C <sub>21</sub> H <sub>22</sub> O <sub>4</sub>                | 11.60908 | ✓               |        |        |        | Chalcone        |
| 66 | Dibutyl sebacate                         | C <sub>18</sub> H <sub>34</sub> O <sub>4</sub>                | 11.64977 | ✓               |        |        | ✓      | Ester           |
| 67 | Semiaquilegoside A                       | C <sub>26</sub> H <sub>38</sub> O <sub>9</sub>                | 11.66309 | ✓               |        |        |        | Glycoside       |
| 68 | Simalikalactone D                        | C <sub>25</sub> H <sub>34</sub> O <sub>9</sub>                | 11.67159 |                 |        | ✓      |        | Triterpenoid    |
| 69 | trans-Octadecyl-ferulate                 | C <sub>28</sub> H <sub>46</sub> O <sub>4</sub>                | 11.82318 | ✓               | ✓      | ✓      | ✓      | Ester           |
| 70 | Isorhamnetin-3-O-β-gentiobioside         | C <sub>28</sub> H <sub>32</sub> O <sub>17</sub>               | 12.02058 | ✓               |        |        |        | Glycoside       |
| 71 | Vaccaroside C                            | C <sub>60</sub> H <sub>96</sub> O <sub>30</sub>               | 12.02091 | ✓               |        |        |        | Glycoside       |
| 72 | Lobetyolinin                             | C <sub>26</sub> H <sub>38</sub> O <sub>13</sub>               | 12.02325 | ✓               |        |        |        | Glycoside       |
| 73 | 3-O-Decanoyl-16-O-acetylisoiridogermanal | C <sub>42</sub> H <sub>70</sub> O <sub>6</sub>                | 12.13242 | ✓               |        |        |        | Triterpenoid    |
| 74 | Methyl octadecenoate                     | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>                | 12.22369 | ✓               | ✓      |        |        | Ester           |
| 75 | Cerevisterol                             | C <sub>28</sub> H <sub>46</sub> O <sub>3</sub>                | 12.32243 | ✓               | ✓      |        |        | Steroid         |
| 76 | Hordatine B                              | C <sub>29</sub> H <sub>40</sub> N <sub>8</sub> O <sub>5</sub> | 12.32865 | ✓               |        |        |        | Alkaloid        |
| 77 | Lablaboside C                            | C <sub>60</sub> H <sub>96</sub> O                             | 12.40509 | ✓               |        |        |        | Glycoside       |
| 78 | Grayanotoxin I                           | C <sub>22</sub> H <sub>36</sub> O <sub>7</sub>                | 12.43372 | ✓               |        |        |        | Terpenoid       |
| 79 | Noadecanoic acid                         | C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>                | 12.44144 | ✓               | ✓      | ✓      | ✓      | Carboxylic acid |
| 80 | Magnocurarine                            | C <sub>19</sub> H <sub>24</sub> NO <sub>3</sub>               | 12.56610 | ✓               | ✓      | ✓      | ✓      | Alkaloid        |
| 81 | Lucidenic acid A                         | C <sub>27</sub> H <sub>38</sub> O <sub>6</sub>                | 12.61707 | ✓               | ✓      |        |        | Triterpenoid    |
| 82 | 2"-O-Rhamno-sylcariside II               | C <sub>33</sub> H <sub>40</sub> O <sub>14</sub>               | 12.73850 | ✓               |        |        |        | Glycoside       |
| 83 | Coixenolide                              | C <sub>38</sub> H <sub>70</sub> O <sub>4</sub>                | 12.83094 | ✓               | ✓      | ✓      | ✓      | Ester           |
| 84 | 16-Methyl-heptadeca-noic acid            | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>                | 12.84120 | ✓               | ✓      |        | ✓      | Carboxylic acid |
| 85 | Sibiricaxanthone A                       | C <sub>24</sub> H <sub>26</sub> O <sub>14</sub>               | 12.87452 |                 |        | ✓      | ✓      | Xanthone        |
| 86 | Ginsenoside Rh4                          | C <sub>36</sub> H <sub>60</sub> O <sub>8</sub>                | 12.92610 | ✓               | ✓      |        |        | Saponin         |
| 87 | Adhyperforin                             | C <sub>36</sub> H <sub>54</sub> O <sub>4</sub>                | 13.05406 | ✓               |        |        |        | Terpenoid       |
| 88 | Zhebeinone                               | C <sub>27</sub> H <sub>43</sub> NO <sub>3</sub>               | 13.05423 | ✓               |        |        |        | Alkaloid        |
| 89 | Notoginsenoside G                        | C <sub>48</sub> H <sub>80</sub> O <sub>19</sub>               | 13.07328 | ✓               |        |        |        | Glycoside       |
| 90 | Syringaresinolmono-β-D-glucoside         | C <sub>34</sub> H <sub>46</sub> O <sub>18</sub>               | 13.16850 |                 |        | ✓      |        | Glycoside       |
| 91 | Jujuboside B                             | C <sub>52</sub> H <sub>84</sub> O <sub>21</sub>               | 13.16942 | ✓               |        |        |        | Glycoside       |
| 92 | Phytolaccagenic acid                     | C <sub>31</sub> H <sub>48</sub> O <sub>6</sub>                | 13.28840 | ✓               | ✓      | ✓      | ✓      | Triterpenoid    |
| 93 | Gypenoside XVII                          | C <sub>48</sub> H <sub>82</sub> O <sub>18</sub>               | 13.53262 | ✓               |        |        |        | Glycoside       |
| 94 | Piperolactam-C9:1(8E)                    | C <sub>20</sub> H <sub>27</sub> NO <sub>3</sub>               | 13.75652 | ✓               |        | ✓      | ✓      | Alkaloid        |
| 95 | Notoginsenoside R2                       | C <sub>41</sub> H <sub>70</sub> O <sub>13</sub>               | 14.26874 | ✓               |        |        |        | Glycoside       |
| 96 | Angeloylgomisin O                        | C <sub>28</sub> H <sub>34</sub> O <sub>8</sub>                | 14.38613 | ✓               |        |        |        | Lignan          |
| 97 | Epimedin C                               | C <sub>39</sub> H <sub>50</sub> O <sub>19</sub>               | 14.51583 | ✓               |        |        |        | Flavonoid       |
| 98 | Protostemotinine                         | C <sub>23</sub> H <sub>29</sub> NO <sub>6</sub>               | 14.54077 | ✓               |        |        |        | Alkaloid        |

**Table 1** (continued)

| No  | Compounds                            | Formular                                                      | RT(min)  | Butanol extract | SubF2b | SubF2d | SubF5b | Classification  |
|-----|--------------------------------------|---------------------------------------------------------------|----------|-----------------|--------|--------|--------|-----------------|
| 99  | Gossypitrin                          | C <sub>21</sub> H <sub>20</sub> O <sub>13</sub>               | 14.92102 | ✓               |        |        | ✓      | Glycoside       |
| 100 | Sulfoorientalol C                    | C <sub>15</sub> H <sub>24</sub> O <sub>4</sub> S              | 14.92234 | ✓               |        |        |        | Terpenoid       |
| 101 | Sanggenon C                          | C <sub>40</sub> H <sub>36</sub> O <sub>12</sub>               | 14.93788 | ✓               | ✓      |        | ✓      | Flavonoid       |
| 102 | Eclalbasaponin IX                    | C <sub>36</sub> H <sub>62</sub> O <sub>11</sub> S             | 14.98683 | ✓               | ✓      |        |        | Triterpenoid    |
| 103 | Physanol A                           | C <sub>36</sub> H <sub>50</sub> O <sub>4</sub>                | 15.24335 | ✓               | ✓      | ✓      | ✓      | Steroid         |
| 104 | Cumambrin A                          | C <sub>17</sub> H <sub>22</sub> O <sub>5</sub>                | 15.80233 | ✓               |        |        |        | Terpenoid       |
| 105 | Physanol B                           | C <sub>36</sub> H <sub>52</sub> O <sub>4</sub>                | 16.20981 | ✓               | ✓      | ✓      | ✓      | Steroid         |
| 106 | 12-Methyl-tetradeca-noic acid        | C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>                | 16.22126 | ✓               |        |        | ✓      | Carboxylic acid |
| 107 | Ostruthin                            | C <sub>19</sub> H <sub>22</sub> O <sub>3</sub>                | 16.26038 |                 |        |        | ✓      | Coumarin        |
| 108 | Phytolacca cerebroside               | C <sub>48</sub> H <sub>93</sub> NO <sub>10</sub>              | 16.33878 |                 |        | ✓      | ✓      | Alkaloid        |
| 109 | Bilirubin                            | C <sub>33</sub> H <sub>36</sub> N <sub>4</sub> O <sub>6</sub> | 16.38911 | ✓               | ✓      |        | ✓      | Alkaloid        |
| 110 | Neohecogenin-3-O-β-D-glucopyranoside | C <sub>33</sub> H <sub>52</sub> O <sub>9</sub>                | 16.53351 | ✓               | ✓      | ✓      | ✓      | Saponin         |
| 111 | 1-Vinyl-4-methoxy-β-carboline        | C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O              | 16.53398 | ✓               | ✓      | ✓      | ✓      | Alkaloid        |
| 112 | Tenacissoside L                      | C <sub>42</sub> H <sub>72</sub> O <sub>16</sub>               | 16.56521 | ✓               |        |        |        | Glycoside       |
| 113 | Limonin                              | C <sub>26</sub> H <sub>30</sub> O <sub>8</sub>                | 16.69495 | ✓               |        |        |        | Triterpenoid    |
| 114 | Acetylcorynoline                     | C <sub>23</sub> H <sub>23</sub> NO <sub>6</sub>               | 16.81192 |                 |        | ✓      |        | Alkaloid        |

**Table 2** The high-abundance compounds of SubF5b with anticancer activity

| Component name                | Molecular formula                                             | Structure                                                                           | Molecular weight (g/mol) | Cancer cells                                  | Pathways                                                     | References |
|-------------------------------|---------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------|-----------------------------------------------|--------------------------------------------------------------|------------|
| Brucine                       | C <sub>23</sub> H <sub>26</sub> N <sub>2</sub> O <sub>4</sub> |  | 394.5                    | ME-180 cervical cancer cell                   | Apoptosis via PI3K/AKT/mTOR pathway                          | [39]       |
| 16-Methyl-heptadeca-noic acid | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>                |  | 284.5                    | KB oral cancer cell and A431 skin cancer cell | Apoptosis via ROS pathway                                    | [40]       |
| Gossypitrin                   | C <sub>21</sub> H <sub>20</sub> O <sub>13</sub>               |  | 480.4                    | Ca9-22 oral squamous cell carcinoma           | Cycle arrest at the G2/M phase and factor-kappa B activation | [41]       |
| Physanol B                    | C <sub>28</sub> H <sub>30</sub> O <sub>9</sub>                |  | 510.5                    | HGC-27 and SGC-7901 gastric cancer cells      | G0/G1 arrest and caspase-dependent apoptosis                 | [42]       |

Inhibition of these proteins has been demonstrated to promote cancer cell senescence [47]. These findings establish CDK4/CDK6 targeting as a potential therapeutic strategy against cancer cell proliferation [48].

Our real-time PCR analysis provided evidence that SubF5b modulates key regulators of the cell cycle and cellular senescence in gastric cancer MKN45 cells. A significant downregulation of cell cycle-promoting genes

(CDK4, CDK6, and CDK8) was observed in SubF5b-treated cells compared to controls. This transcriptional suppression correlates with the previously demonstrated G0/G1 cell cycle arrest via flow cytometry. Concurrently, the marked upregulation of P21 and P16, two well characterized cyclin dependent kinase inhibitors, further supports the hypothesis that SubF5b activates pro-senescence signaling pathways in MKN45 cells.

P21 is widely recognized for its dual role in enforcing cell cycle arrest [49] and inducing cancer cell senescence [47, 48] in response to stress stimuli. The ~sevenfold increase in P21 mRNA coupled with 12-fold elevated P21 protein underscores its central role in SubF5b-mediated growth inhibition. Similarly, P16 induction (albeit to a lesser extent) reinforced the senescence phenotype. Conversely, Ki67 and PCNA—established proliferation markers [50] showed significant reduction at both transcriptional and protein levels. The diminished expression of these markers serves as a critical indicator of cellular senescence [51, 52]. Our data revealed >tenfold reduction in Ki67 mRNA and 20-fold decrease in Ki67 protein, demonstrating potent antiproliferative activity of SubF5b through senescence induction.

These findings align with previous studies showing that natural compounds (e.g., Platycodin D2, curcumin, epigallocatechin gallate, berberine, and piperlongumine) can trigger cancer cell senescence via P21 upregulation and cell cycle regulator suppression [50, 53]. SubF5b appears to coordinate a concerted transcriptional and translational response that suppresses proliferation and induces senescence through the CDK-P21 axis. The upregulation of cell cycle inhibitory proteins such as P21

and P16, along with the downregulation of proliferation markers including Ki67 and PCNA, indicates a multi-targeted effect of the compounds present in SubF5b. The downstream mechanisms may involve increased production of reactive oxygen species (ROS), leading to the activation of apoptotic signaling pathways and induction of cell cycle arrest [54]. Furthermore, recent studies have demonstrated that compounds derived from *Ardisia* extracts exert anticancer effects by downregulating cancer stem cell-associated signaling pathways [12, 55]. The data presented in this study have demonstrated the anticancer potential of the butanol subfractions of the *Ardisia gigantifolia* through the mechanisms of cell cycle arrest and induction of cellular senescence via in vitro experiments (Fig. 7). The absence of toxicity evaluations of SubF5b in animal models is acknowledged as a major limitation of the present study and should be addressed in future investigations to support its potential for clinical application. In addition, the use of extract fractions containing multiple compounds is well recognized in traditional Chinese medicine. However, crude mixtures may pose risks such as unintended toxicity from unidentified compounds, low bioavailability, and batch-to-batch variability. These factors could undermine stability and reproducibility in therapeutic use. Furthermore, the present study lacks pharmacokinetic and stability evaluations, which are also considered limitations. Therefore, future studies should focus on isolating key compounds, conducting in vivo toxicity assessments, performing pharmacokinetic and stability evaluations, and developing standardized sample preparation protocols prior

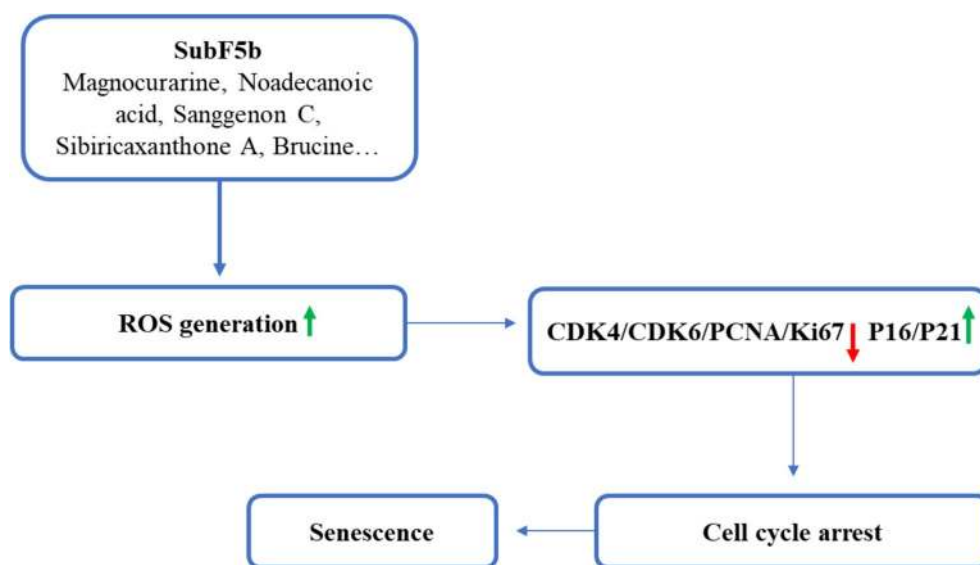


**Fig. 7** Proposed anticancer mechanism of SubF5b in gastric cancer cells

to advancing toward preclinical testing and clinical application.

## Conclusion

This study assessed the phytochemical composition and anticancer potential of the butanol extract and its subfractions derived from *Ardisia gigantifolia* leaves. UPLC-QToF-MS analysis identified 103 compounds in the crude extract and 33–35 compounds per subfraction, with distinct compositional patterns implying functional specialization. Sanggenon C, Xanthones, Brucine, and Coixenolide are metabolites that have been reported to possess anticancer activity and are identified for the first time in the extract of *Ardisia gigantifolia*. SubF5b demonstrated the most potent antiproliferative effects against the MCF-7, MKN45, and AGS cell lines, as evidenced by significantly lower IC<sub>50</sub> values compared to other fractions. Mechanistic investigations revealed that SubF5b induced G0/G1 cell cycle arrest and senescence, as confirmed by  $\beta$ -galactosidase activity and differential expression of markers related to senescence and the cell cycle. SubF5b was found to downregulate CDK4/6/8, Ki67, and PCNA, while upregulating P21 and P16 at both the mRNA and protein levels, indicating its dual role in inhibiting the cell cycle and activating senescence. These findings suggest that SubF5b is a promising plant-derived candidate for anticancer therapy, targeting cell cycle dysregulation and pro-senescence pathways.

## Abbreviations

|              |                                                                                     |
|--------------|-------------------------------------------------------------------------------------|
| CCNB2        | Cyclin B2                                                                           |
| CCNE1        | Cyclin E1                                                                           |
| CCND1        | Cyclin D1                                                                           |
| CCND2        | Cyclin D2                                                                           |
| CDK          | Cyclin-dependent kinase                                                             |
| CDK2         | Cyclin-dependent kinase 2                                                           |
| CDK3         | Cyclin-dependent kinase 3                                                           |
| CDK4         | Cyclin-dependent kinase 4                                                           |
| CDK6         | Cyclin-dependent kinase 6                                                           |
| CDK8         | Cyclin-dependent kinase 8                                                           |
| PCNA         | Proliferating cell nuclear antigen                                                  |
| SubF5b       | Subfraction 5b                                                                      |
| SubF2b       | Subfraction 2b                                                                      |
| SubF2d       | Subfraction 2d                                                                      |
| DMSO         | Dimethyl sulfoxide                                                                  |
| MTT          | 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide                        |
| UPLC-QToF-MS | Ultra performance liquid chromatography-quadrupole time of flight mass spectrometry |
| RPMI1640     | Roswell Park Memorial Institute 1640                                                |

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-026-00971-2>.

Supplementary Material

## Acknowledgements

Not applicable.

## Author contributions

Nguyet Mai Hua, Van Khang Pham: Data curation, Formal analysis, Writing—original draft, Funding acquisition. Phu Hung Nguyen, Conceptualization, Writing—original draft, Project administration, Resources. Van Hung Hoang, Thi Thanh Huong Le: Writing—review & editing. Thi Kieu Oanh Nguyen, Son Hiep Pham, Hong Phuong Ngo: Methodology, Investigation, Data curation, Formal analysis.

## Funding

This study was financially supported by the research project CS2024-TN06-014 funded by Thai Nguyen University of Sciences, and the research project DH2024-NV-02 funded by Thai Nguyen University.

## Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Competing interests

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Author details

<sup>1</sup>TNU- University of Sciences (TNUS), Thai Nguyen City 24000, Vietnam.

<sup>2</sup>TNU - University of Education (TNUE), Thai Nguyen City 24000, Vietnam.

<sup>3</sup>Institute for Excellence in Education and Research, Thai Nguyen University, Thai Nguyen 24000, Vietnam. <sup>4</sup>University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam.

Received: 19 May 2025 Accepted: 19 March 2026

Published online: 27 March 2026

## References

1. Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A (2025) Cancer statistics, 2025. *CA Cancer J Clin* 75:10–45. <https://doi.org/10.3322/caac.21871>
2. The global challenge of cancer (2020) *Nat Cancer* 1:1–2. <https://doi.org/10.1038/s43018-019-0023-9>
3. Tilaoui M, Ait Mouse H, Ziyad A (2021) Update and new insights on future cancer drug candidates from plant-based alkaloids. *Front Pharmacol* 12:719694. <https://doi.org/10.3389/fphar.2021.719694>
4. Cech NB, Oberlies NH (2023) From plant to cancer drug: lessons learned from the discovery of taxol. *Nat Prod Rep* 40:1153–1157. <https://doi.org/10.1039/D3NP00017F>
5. Sati P, Sharma E, Dhyani P, Attri DC, Rana R, Kiyekbayeva L, Büsselberg D, Samuel SM, Sharifi-Rad J (2024) Paclitaxel and its semi-synthetic derivatives: comprehensive insights into chemical structure, mechanisms of action, and anticancer properties. *Eur J Med Res* 29:90. <https://doi.org/10.1186/s40001-024-01657-2>
6. Liu B, Liu R, Liu Q, Ashby CR, Zhang H, Chen Z (2022) The ethnomedicinal and functional uses, phytochemical and pharmacology of compounds from *Ardisia* species: an updated review. *Med Res Rev* 42:1888–1929. <https://doi.org/10.1002/med.21894>
7. Yu J, Qu S, Xie Q, Shama R, Bao M, Su H, Liu B, Borjigidai A (2023) *Ardisia gigantifolia* stapf (Primulaceae): a review of ethnobotany, phytochemistry, pharmacology, clinical application, and toxicity. *J Ethnopharmacol* 305:116079. <https://doi.org/10.1016/j.jep.2022.116079>
8. Wen P, Zhang X-M, Yang Z, Wang N-L, Yao X-S (2008) Four new triterpenoid saponins from *Ardisia gigantifolia* Stapf. and their cytotoxic activity. *J Asian Nat Prod Res* 10:873–880. <https://doi.org/10.1080/10286020802144719>
9. Mu L-H, Wang L-H, Yu T-F, Wang Y-N, Yan H, Liu P, Yan C (2020) Triterpenoid saponin AG8 from *Ardisia gigantifolia* stapf. induces Triple Negative Breast

- Cancer cells apoptosis through oxidative stress pathway. *Oxid Med Cell Longev* 2020:1–11. <https://doi.org/10.1155/2020/7963212>
10. Bai N, Wang Q, Zhang C, Wang J (2024) Triterpenoid saponin from *Ardisia gigantifolia* Stapf inhibits cancer cell metastasis behavior of TNBC via suppressing EMT and stemness through ITGB4/FAK signals. *Clin Tradit Med Pharmacol* 5:200182. <https://doi.org/10.1016/j.ctmp.2024.200182>
  11. Dai W, Yang J, Liu X, Mei Q, Peng W, Hu X (2023) Anti-colorectal cancer of *Ardisia gigantifolia* Stapf. and targets prediction via network pharmacology and molecular docking study. *BMC Complement Med Ther* 23:4. <https://doi.org/10.1186/s12906-022-03822-8>
  12. Le TT, Nguyen PH, Nguyen VP, Nguyen TN (2023) *Ardisia gigantifolia* ethanolic extract inhibits cell proliferation and targets cancer stem cells in gastric cancer. *Asian Pac J Trop Biomed* 13:258–267. <https://doi.org/10.4103/2221-1691.378600>
  13. Le TTH, Ngo TH, Nguyen TH, Hoang VH, Nguyen VH, Nguyen PH (2023) Anti-cancer activity of green synthesized silver nanoparticles using *Ardisia gigantifolia* leaf extract against gastric cancer cells. *Biochem Biophys Res Commun* 661:99–107. <https://doi.org/10.1016/j.bbrc.2023.04.037>
  14. Majinda RRT (2012) Extraction and isolation of saponins. In: Sarker SD, Nahar L (eds) *Natural products isolation*. Humana Press, Totowa, pp 415–426. [https://doi.org/10.1007/978-1-61779-624-1\\_16](https://doi.org/10.1007/978-1-61779-624-1_16)
  15. Nguyen PH, Le TTH, Can DQH, Le TNT, Hoang V, Ngo HP, Do TTH, Nguyen TKO, Hoang VH (2025) Anticancer potential of *Ampelopsis cantoniensis* extract: inducing apoptosis, cell cycle arrest, and targeting cancer stem cells in HepG2 liver cancer cells. *Biocatal Agric Biotechnol* 64:103521. <https://doi.org/10.1016/j.bcab.2025.103521>
  16. Van Hao N, Tung DH, Hung NP, Hoa VX, Ha NT, Van Khanh NT, Tan PT, Van Trinh P (2023) Green, facile and fast synthesis of silver nanoparticles by using solution plasma techniques and their antibacterial and anticancer activities. *RSC Adv* 13:21838–21849. <https://doi.org/10.1039/D3RA03454B>
  17. Le Huong TT, Nguyen MQ, Nguyen VP, Hoang VH, Nguyen PH (2023) Methanol extract of *Ficus pumila* L. inhibits proliferation, induces apoptosis and arrests the cell cycle in HepG2 live cancer cells. *Asian J Plant Sci* 22:423–433. <https://doi.org/10.3923/ajps.2023.423.433>
  18. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-ΔΔCT</sup> method. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>
  19. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C (2023) Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. *Front Pharmacol* 14:1265178. <https://doi.org/10.3389/fphar.2023.1265178>
  20. Fukuzawa M, Yamaguchi R, Hide I, Chen Z, Hirai Y, Sugimoto A, Yasuhara T, Nakata Y (2008) Possible involvement of long chain fatty acids in the spores of *Ganoderma lucidum* (Reishi Houshi) to its anti-tumor activity. *Biol Pharm Bull* 31:1933–1937. <https://doi.org/10.1248/bpb.31.1933>
  21. Okon E, Kukula-Koch W, Halasa M, Jarzab A, Baran M, Dmoszynska-Graniczka M, Angelis A, Kalpoutzakis E, Guz M, Stepulak A, Wawruszak A (2020) Magnoflorine—isolation and the anticancer potential against NCI-H1299 lung, MDA-MB-468 breast, T98G glioma, and TE671 rhabdomyosarcoma cancer cells. *Biomolecules* 10:1532. <https://doi.org/10.3390/biom10111532>
  22. Saleri F, Chen G, Li X, Guo M (2017) Comparative analysis of saponins from different *Phytolaccaceae* species and their antiproliferative activities. *Molecules* 22:1077. <https://doi.org/10.3390/molecules22071077>
  23. AlAlawi RA, Hoheisel JD, Alhamdani MSS, Baqi Y (2025) *Phoenix dactylifera* L. (date palm) fruit extracts and fractions exhibit anti-proliferative activity against human pancreatic cancer cell lines. *Heliyon* 11:e42274. <https://doi.org/10.1016/j.heliyon.2025.e42274>
  24. Qin Y, Zhou R, Jin J, Xie J, Liu H, Cai P, Shu J, Zhao Y, Huang L, Zhang S (2020) UPLC–ESI–Q–TOF–MS/MS analysis of anticancer fractions from *Ophiocordyceps xuefengensis* and *Ophiocordyceps sinensis*. *Biomed Chromatogr* 34:e4841. <https://doi.org/10.1002/bmc.4841>
  25. Roy A, Anand A, Garg S, Khan MS, Bhasin S, Asghar MN, Emran TB (2022) Structure-based in silico investigation of agonists for proteins involved in breast cancer. *Evid Based Complement Alternat Med* 2022:1–12. <https://doi.org/10.1155/2022/7278731>
  26. Wang T, Qi D, Hu X, Li N, Zhang X, Liu H, Zhong C, Zhang J (2021) A novel evodiamine amino derivative as a PI3K/AKT signaling pathway modulator that induces apoptosis in small cell lung cancer cells. *Eur J Pharmacol* 906:174215. <https://doi.org/10.1016/j.ejphar.2021.174215>
  27. Chen J, Liu B-X, Shen Q, Li N, Ling J, Xiao M, Jiao H-Y, Li T (2020) Limonin inhibits angiogenesis and metastasis of human breast cancer cells by suppressing the VEGFR2/IGF1-mediated STAT3 signaling pathway. *Transl Cancer Res* 9:6820–6832. <https://doi.org/10.21037/tcr-20-1992>
  28. Fagbohun OF, Olawoye B, Ademakinwa AN, Oriyomi OV, Fagbohun OS, Fadare OA, Msagati TAM (2021) UHPLC/GC-TOF-MS metabolomics, MTT assay, and molecular docking studies reveal physostigmine as a new anticancer agent from the ethyl acetate and butanol fractions of *Kigelia africana*. (Lam.) Benth fruit extracts. *Biomed Chromatogr* 35:e4979. <https://doi.org/10.1002/bmc.4979>
  29. Nordin ML, Kadir AA, Zakaria ZA, Abdullah R, Abdullah MNH (2018) In vitro investigation of cytotoxic and antioxidative activities of *Ardisia crissa* against breast cancer cell lines, MCF-7 and MDA-MB-231. *BMC Complement Altern Med* 18:87. <https://doi.org/10.1186/s12906-018-2153-5>
  30. Dai W, Yang J, Liu X, Mei Q, Peng W, Hu X (2023) Anti-colorectal cancer of *Ardisia gigantifolia* Stapf. and targets prediction via network pharmacology and molecular docking study. *BMC Complement Med Ther* 23:4. <https://doi.org/10.1186/s12906-022-03822-2>
  31. Aipire A, Chen Q, Cai S, Li J, Fu C, Ying T, Lu J, Li J (2018) N-Butanol subfraction of *Brassica rapa* L. promotes reactive oxygen species production and induces apoptosis of A549 lung adenocarcinoma cells via mitochondria-dependent pathway. *Molecules* 23:1687. <https://doi.org/10.3390/molecules23071687>
  32. Zhang N, Zhao L, Cai S, Zeng X, Wu W, Ji B, Zhou F (2021) Ethyl acetate subfractions from ethanol extracts of fermented oats (*Avena sativa* L.) exert anti-cancer properties in vitro and in vivo through G2/M and S Phase arrest and apoptosis. *J Cancer* 12:1853–1866. <https://doi.org/10.7150/jca.48993>
  33. Kumari R, Jat P (2021) Mechanisms of cellular senescence: cell cycle arrest and senescence associated secretory phenotype. *Front Cell Dev Biol* 9:645593. <https://doi.org/10.3389/fcell.2021.645593>
  34. Hoang VH, Le TTH, Nguyen PH, Nguyen DH, Luc QT, Nguyen TKO, Do KH (2025) Anticancer activity of *Elsholtzia winitiana* var. *dongvanensis* Phuon on gastric cancer cells: phytochemical profiling, cell proliferation inhibition, senescence induction, and cell cycle arrest. *Beni-Suef Univ J Basic Appl Sci* 14:39. <https://doi.org/10.1186/s43088-025-00634-w>
  35. Kaewtunjai N, Wongpoomchai R, Imsumran A, Pompimon W, Athipornchai A, Suksamrarn A, Lee TR, Tuntiwechapikul W (2018) Ginger extract promotes telomere shortening and cellular senescence in A549 lung cancer cells. *ACS Omega* 3:18572–18581. <https://doi.org/10.1021/acsomega.8b02853>
  36. Erceli K M, Tunca B, Aksoy SA, Aksoy CA, Tezcan G (2023) *Olea europaea* L. Leaf extract attenuates temozolomide-induced senescence-associated secretion phenotype in glioblastoma. *Tjps* 20:68–77. <https://doi.org/10.4274/tjps.galenos.2022.57639>
  37. Song YS, Lee BY, Hwang ES (2005) Distinct ROS and biochemical profiles in cells undergoing DNA damage-induced senescence and apoptosis. *Mech Ageing Dev* 126:580–590. <https://doi.org/10.1016/j.mad.2004.11.008>
  38. Wang L, Lankhorst L, Bernards R (2022) Exploiting senescence for the treatment of cancer. *Nat Rev Cancer* 22:340–355. <https://doi.org/10.1038/s41568-022-00450-9>
  39. Seshadri VD (2021) Brucine promotes apoptosis in cervical cancer cells (ME-180) via suppression of inflammation and cell proliferation by regulating PI3K / AKT / mTOR signaling pathway. *Environ Toxicol* 36:1841–1847. <https://doi.org/10.1002/tox.23304>
  40. Saravanakumar K, Vivek R, Boopathy NS, Yaqian L, Kathiresan K, Chen J (2015) Anticancer potential of bioactive 16-methylheptadecanoic acid methyl ester derived from marine *Trichoderma*. *J Appl Biomed* 13:199–212. <https://doi.org/10.1016/j.jab.2015.04.001>
  41. Fang C, Chen C, Yang Y, Li K, Gao R, Xu D, Huang Y, Chen Z, Liu Z, Chen S, Yu X, Li Y, Zeng C (2022) Physalin B inhibits cell proliferation and induces apoptosis in undifferentiated human gastric cancer HGC-27 cells. *Asia-Pac J Clin Oncol* 18:224–231. <https://doi.org/10.1111/ajco.13593>
  42. Huang K, Liu Z, Kim M-O, Kim K-R (2023) Anticancer effects of gossypetin from *Hibiscus sabdariffa* in oral squamous cell carcinoma. *J Appl Oral Sci* 31:e20230243. <https://doi.org/10.1590/1678-7757-2023-0243>
  43. Liu J (2011) Sanggenon C decreases tumor cell viability associated with proteasome inhibition. *Front Biosci* E3:1315–1325. <https://doi.org/10.2741/e335>

44. Li J, Cao X, Chu T, Lin K, Chen L, Lv J, Tan Y, Chen M, Li M, Wang K, Zheng Q, Li D (2023) The circHMGCS1-miR-205-5p-ErBB3 axis mediated the Sanggenon C-induced anti-proliferation effects on human prostate cancer. *Pharmacol Res* 187:106584. <https://doi.org/10.1016/j.phrs.2022.106584>
45. Matsumoto K, Akao Y, Ohguchi K, Ito T, Tanaka T, Iinuma M, Nozawa Y (2005) Xanthenes induce cell-cycle arrest and apoptosis in human colon cancer DLD-1 cells. *Bioorg Med Chem* 13:6064–6069. <https://doi.org/10.1016/j.bmc.2005.06.065>
46. Kim S, Leong A, Kim M, Yang HW (2022) CDK4/6 initiates Rb inactivation and CDK2 activity coordinates cell-cycle commitment and G1/S transition. *Sci Rep* 12:16810. <https://doi.org/10.1038/s41598-022-20769-5>
47. Crozier L, Foy R, Adib R, Kar A, Holt JA, Pareri AU, Valverde JM, Rivera R, Weston WA, Wilson R, Regnault C, Whitfield P, Badonyi M, Bennett LG, Vernon EG, Gamble A, Marsh JA, Staples CJ, Saurin AT, Barr AR, Ly T (2023) CDK4/6 inhibitor-mediated cell overgrowth triggers osmotic and replication stress to promote senescence. *Mol Cell* 83:4062–4077.e5. <https://doi.org/10.1016/j.molcel.2023.10.016>
48. Sun M, Dong L, Wang Y, Liu C, Du J, Wang B, Xing B, Yao X, Ren Y, Zhou X (2024) The role of targeting CDK4/6 in cancer immunotherapy. *Holistic Integr Oncol* 3:32. <https://doi.org/10.1007/s44178-024-00100-0>
49. Karimian A, Ahmadi Y, Yousefi B (2016) Multiple functions of p21 in cell cycle, apoptosis and transcriptional regulation after DNA damage. *DNA Repair (Amst)* 42:63–71. <https://doi.org/10.1016/j.dnarep.2016.04.008>
50. Juríková M, Danihel L, Polák Š, Varga I (2016) Ki67, PCNA, and MCM proteins: markers of proliferation in the diagnosis of breast cancer. *Acta Histochem* 118:544–552. <https://doi.org/10.1016/j.acthis.2016.05.002>
51. Mansour MA, Rahman M, Ayad AA, Warrington AE, Burns TC (2023) P21 overexpression promotes cell death and induces senescence in human Glioblastoma. *Cancers (Basel)* 15:1279. <https://doi.org/10.3390/cancers15041279>
52. Niklasson M, Dalmo E, Segerman A, Rendo V, Westermarck B (2025) p21-dependent senescence induction by BMP4 renders Glioblastoma cells vulnerable to senolytics. *Int J Mol Sci* 26:3974. <https://doi.org/10.3390/ijms26093974>
53. Correia-Melo C, Jurk D, Passos JF (2013) Robust multiparametric assessment of cellular senescence. In: Galluzzi L, Vitale I, Kepp O, Kroemer G (eds) *Cell senescence*. Humana Press, Totowa, pp 409–419. [https://doi.org/10.1007/978-1-62703-239-1\\_27](https://doi.org/10.1007/978-1-62703-239-1_27)
54. Mu L-H, Yan H, Wang Y-N, Yu T-F, Liu P (2019) Triterpenoid saponins from *Ardisia gigantifolia* and mechanism on inhibiting proliferation of MDA-MB-231 cells. *Biol Pharm Bull* 42:194–200. <https://doi.org/10.1248/bpb.b18-00569>
55. Sun L, Li Y, Zhao R, Fan Q, Liu F, Zhu Y, Han J, Liu Y, Jin N, Li X, Li Y (2024) Platycodin D2 enhances P21/CyclinA2-mediated senescence of HCC cells by regulating NIX-induced mitophagy. *Cancer Cell Int* 24:79. <https://doi.org/10.1186/s12935-024-03263-y>

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.